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EXAMINER	
NOBLE, MARCIA STEPHENS	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 05/22/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/507,384	BAETSCHER ET AL.	
	Examiner	Art Unit	
	Marcia S. Noble	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *10-12 and 15* is/are pending in the application.

4a) Of the above claim(s) *1-9,13,14 and 16-27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *10-12 and 15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/7/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of Group VI, claims 10-12 and 15, in the reply filed on 5/2/06 is acknowledged.

Claims 1-9, 13, 14, and 16-27 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected subject matter, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 5/2/06.

Claims 10-12 and 15 are under consideration.

Priority

2. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant claims priority to Provisional application number 60/365,022 (f.d. 3/12/2002). Applicant has complied with conditions for receiving the benefit of an earlier filing date.

Information Disclosure Statement

3. The information disclosure statement filed 1/7/05 fails to comply with 37 CFR 1.98(a)(2), which requires a legible copy of each cited foreign patent document; each non-patent literature publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. It has been placed in the application file, but the information referred to therein has not been considered.

No copies of references 3, 7, 11, 19-21, 24, 26, 28, 29, and 32 were provided. Therefore, these references were crossout and not considered.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

4. Claims 10-12 and 15 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make or use the claimed invention, if not, whether an artisan would require undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of

working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue".

Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification, therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of invention, therefore skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The instant invention is drawn to a method of isolating stem cells comprising: (a) isolating a blastocyst; (b) identifying those cells which rely upon glycolysis for survival; (c) isolating a glycolytic cell from the inner cell mass of the blastocyst; (d) culturing the isolated glycolytic cells to obtain an isolated stem cell. Narrowing embodiments specify the cells are identified by staining with JC-1 and that the method further comprise maintaining the isolated cells on a fibroblast feeder layer to prevent differentiation.

The invention is also drawn to a method of producing glycolytic-dependent cells comprising: (a) culturing cells under hypoxic conditions, (b) identifying those cells which rely upon glycolysis for survival; (c) isolating the glycolytic cells from culture, and culturing the isolated glycolytic cells.

The specification discloses that isolated stem cells are sustainable under glycolytic conditions and maintain the potential to differentiate (p. 5, lines 7-9). Under both differentiating (-LIF) or undifferentiating (+LIF) culture conditions two subpopulations of ES cells can be detected using mitochondrial membrane potential marker, JC-1. ES cells fluorescing with green, indicting glycolysis, or red, indicating

oxidative phosphorylation, are detected with JC-1 staining. The number of ES cells using oxidative phosphorylation increases when the cells are grown or expanded but maintained in undifferentiated using LIF (p 8-9, [0035]), however, a subpopulation of cells using glycolysis, staining green for JC-1 persists. The specification also discloses that ES cells with high mitochondrial membrane potential (stain red) display low differentiation permissiveness, while ES cells with low mitochondrial membrane potential (stain green) display high differentiation permissiveness (p. 10, lines 10-12). The specification is suggesting that the green staining, glycolytic population of ES cells are less differentiated than the ones staining red, using oxidative phosphorylation and that the glycolytic green cells are more suitable for use in the production of chimeric animals or other stems cell use. The specification discloses a method of culturing mouse ES cells (example 1 p. 13, [0046], using flow cytometry to sort ES cells that stain red or green for mitochondrial membrane potential marker, JC-1 (example 2, p. 13, [0047]), using the sorted cells to produce chimeric mice (p 14-16 and specifically with green cells, example 15, p19 [0062]). The specification also teaches significantly more chimeric mice were produced with the cells that stain green than those that stain red (p. 20, lines 1-2).

However, the method disclosed by the specification is not a method of isolating stem cells as claimed. First, the claimed method utilizes blastocysts to isolate stem cells with the underlying assumption that all blastomeres from the inner cell mass are stem cells.

An ES cell has distinct defining characteristics. ES cells are self-renewing, undifferentiated, are totipotent or plruipotent and have the ability to develop into any cell/tissue type. Hogan et al teach that all the blastomeres in an mouse embryo are equipotent only up to the early-eight cell stage. "Single blastomeres from two and four cell morulae can give rise to a mouse. Early eight-cell-stage blastomeres cannot generate a mouse by themselves, but when recombined with genetically marked morulae they can give rise to a wide range of different tissues in chimeric offspring ... As cleavage proceeds to the 16-cell stage, however, there is a gradual restriction in the developmental potency of the cell." (p. 41, par 1, Manipulating the Mouse Embryo, 1986). Hogan et al also stresses that as the embryo develops through the morula and blastocyst stages, the development of the cells is asynchronous (p. 41, par 2). Therefore, the cells of the developing embryo are differentiating asynchronously as well. Not all cells isolated from the inner cell mass of a blastocyst can develop into any or multiple different tissues nor can all cells isolated from the inner cell develop in a chimera.

However, the instantly claimed method provides no means of isolating the true stem cells from the non-stem cells from the inner cell mass, and therefore the isolating a blastocyst step results in a heterogeneous population of stem cells and non-stem cell. Although not specifically stated in the specification, the disclosed method implies that it was already starting with established stem cells and therefore determining which ones were more or less differentiated.

Second, the next step is identifying those cells which rely on glycolysis for survival. However, all cells have the ability to rely on glycolysis and the instant method of staining with JC-1 is not a measure of survival. The example disclosed in the specification demonstrated that both the red and the green ES cells sorted are surviving because they are both resulting in chimeric animals. The staining is measuring a difference in the amount of glycolysis that is occurring. Therefore an artisan would not know how to use the instantly claimed method nor the method disclosed in the specification to determine which cells rely upon glycolysis.

As stated the method disclosed in the specification is using the JC-1 staining to determine which ES cells in a population of established ES cells are undergoing more glycolysis. However, in the instantly claimed method the starting cells are not an established population of step cells, they are a heterogeneous population of blastomeres from a blastocyst that have different potentials for glycolysis as well. The instant application hypothesizes that glycolysis is a measure for differentiation. However, Diaz et al demonstrate that differentiated, non-stem cells stain green therefore are undergoing glycolysis as well (J Cell Sci 112:1079, Fig 1, 1999). Therefore, the staining does not serve as a dependable marker of differentiation. Furthermore Diaz et al demonstrate that cells can have can have different mitochondrial membrane potential in the same cell demonstrating staining in both green and red with JC-1 (Fig 1 p. 1079 and p. 1077 par 1). This suggests that the marker is not a "black and white" answer for membrane potential status nor glycolytic status. This is consistent with the understanding that a cell will use both pathways simultaneously and

not just one or the other. Also, the claimed method has a heterogeneous population of stem cells and non-stem cells, therefore an artisan would not be able to determine from the claimed method if the cell that are staining green are stem cells undergoing glycolysis or differentiated non-stem cells, therefore, it is not clear that the instant method will isolate stem cells.

The method disclosed in the specification uses the formation of chimeras as an assay for differentiation and the determination of successful isolation of stem cells. However, formation of chimeras is not an assay of differentiation. The formation of a chimera indicates that stem cells were present and were in a state wherein they could develop in a tissue and incorporate into formation of an animal. Although it suggests that stem cells that to some degree are less differentiated were present, it does not determine the level of differentiation nor does it demonstrate its contribution to the animal. It is possible that some of the cells incorporate into the animal but do not divide therefore not contributing to the development of the animal. Overall very little is known about what happen to a ES cell in chimeric formation and at what point an ES cell is not capable of incorporating into an ES cell, therefore, the formation of a chimeric provides little information about the state of differentiation of a cell.

Overall, no clear link between stem cells, differentiation, and glycolysis have been determined established in the art, not does the specification provide a clear link. While the disclosed example of improved numbers of chimeras formed from the use of the green subpopulation with the green staining being an indicator that glycolysis is occurring. The red cells also form chimeras as well, therefore the stems cells are

present in that population and are in a state where they can develop in chimeras as well. Therefore, it is unclear how an artisan will be able to isolate stem cell using the JC-1, glycolytic marker, as a means for identifying and isolating stem cells as claimed.

The instant method also is drawn to a method of glycolytic-dependent cells. This method is broadly drawn to any cell, but still the comments above apply. All cells are dependent on glycolysis to some extent and the JC-1 staining is not a measure of dependence or survival of cells as mentioned above. Furthermore, the cells that are surviving under hypoxic condition and identified as undergoing glycolysis are mostly likely surviving and dependent upon a multitude of factors. The health status and age of the cell could be potentially playing a role as well. Therefore it is not clear that the instant method would be capable of isolating cell dependent on glycolysis.

Overall because a link between glycolysis, cell differentiation, dependence on glycolysis, stem cell status, and chimera formation is not soundly demonstrated in the art or specification, an artisan would not be able to use the instantly claimed invention as a method of isolated stem cells.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 10-12 and 15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 10 recites "isolating a blastocyst". The metes and bound are not clear how isolating a blastocyst will result in the isolating of stem cells. Is the isolation of a blastocyst meaning isolate the cells from a blastocyst. Furthermore, in relation to the other steps it is not clear if the glycolytic cells are supposed to be identified will they are still a part of the blastocyst. Not does the method suggest how the glycolytic cells are to be identifies or isolated from the blastocyst.

Claim 10 recites the limitation "those cells". There is insufficient antecedent basis for this limitation in the claim. Furthermore, the metes and bounds of "those cells" is unclear because to which cells are being referred, the stem cells, the cells of the blastocyst, etc.

Claim 10 recites the limitation "the isolated glycolytic cell" in (d). There is insufficient antecedent basis for this limitation in the claim.

Claim 11 recites the limitation "the cells". There is insufficient antecedent basis for this limitation in the claim.

Claim 11 recites the limitation "the mitochondrial marker JC-1". There is insufficient antecedent basis for this limitation in the claim.

Claim 12 recites the limitation "the isolated cells". There is insufficient antecedent basis for this limitation in the claim.

Claim 15 recites the limitation "those cells". There is insufficient antecedent basis for this limitation in the claim. Furthermore, the metes and bounds of "those cells" is unclear because to which cells are being referred, the stem cells, the cells of the blastocyst, etc.

Claim 15 recites the limitation "the isolated glycolytic cell" in (d). There is insufficient antecedent basis for this limitation in the claim.

Claim 15 recites the limitation "the isolated cells". There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claim 15 is rejected under 35 U.S.C. 102(b) as being anticipated by Papas et al (Biotech Bioeng 66(4): 231-237).

The instant claim is drawn to a method of producing glycolytic-dependent cells, comprising: (a) culturing cells under hypoxic conditions, (b) identifying those cells which rely upon glycolysis for survival; isolating the glycolytic cells from the culture, and (d) culturing the isolated glycolytic cells.

Papas et al discloses a method of culturing encapsulated mouse insulinoma βTC3 cell in reduced oxygen conditions for 3 and 4 days and determined the oxygen consumption rate, glucose consumption rate, and the lactate production rate of these cells. Cells were cultured under two episodes of hypoxic condition (days 11-14 and 19-23 of culture) with a period in between that was not oxygen deprived (p. 232, par bridging col 1 and 2 and col 2 par 1). During periods of hypoxia, the oxygen

consumption rate decreased rapidly at the beginning of the hypoxic period and stabilized at low levels throughout the hypoxic period. Glucose consumption rate and lactate production increased reaching a maxima corresponding to approximately 130 to 150% of their pre-hypoxic level (Fig 1 on p. 233 and p. 232, col 2, par 2 and 3).

Because the βTC3 demonstrated increased lactate production in hypoxic conditions, the method identified them as cell which rely on glycolysis for survival. Because these cells were previously isolated form culture and were continued to be isolated form culture by encapsulation, and were cultured under several episodes of hypoxia with a culture in non-hypoxic conditions, the method isolated the glycolytic cells from culture and the isolate glycolytic cells were subsequently cultured.

7. Claim 15 is rejected under 35 U.S.C. 102(b) as being anticipated by Ferea et al (PNAS 96:9721-9726, 1999).

The instant claim is drawn to a method of producing glycolytic-dependent cells, comprising: (a) culturing cells under hypoxic conditions, (b) identifying those cells which rely upon glycolysis for survival; isolating the glycolytic cells from the culture, and (d) culturing the isolated glycolytic cells.

Ferea et al disclose a method of three long term cultures of yeast under glucose limited media to develop cells that with increased dependency on glycolysis (p. 9721, col 2, par 3). Genetic analysis of the evolved offspring strains of yeast demonstrated alterations and reliance of gene involved in glycolysis (see abstract).

Given the broadest reasonable interpretation, culturing under hypoxic can be considered relative to the cell type and therefore almost any culture condition can be considered oxygen deprived or hypoxic. Because the evolving cells were developed under glucose limited conditions they were dependent upon glycolysis for there survival.

8. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Marcia S. Noble whose telephone number is (571) 272-5545. The examiner can normally be reached on M-F 9 to 5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Marcia S. Noble

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